A correlational study of alcohol consumption and kinship among rats*

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Two experiments involving three generations each of Long-Evans rats are reported. Correlation coefficients (Pearson rs) were calculated among animals having varying degrees of biological kinship, using indices of relative alcohol consumption as data points. No evidence of a relationship between similarity of relative alcohol consumption and closeness of biological kinship was observed.

Several investigators have suggested the existence of a genetically determined alcohol selection behavior in rodents (e.g., Brewster, 1968, 1969; Williams, 1956; Mardones, 1960; Erikssen, 1968, 1969a, b). The most explicit evidence for such a genetic phenomenon lies in the existence of strains of mice such as the C57/BL and the DBA. The presence of a genetic variable associated with alcohol consumption in humans has been investigated by Bleuer (1955), Kaij (1960), and Partanen, Bruun, & Markkanen (1966).

While much of the previous research has suggested the possibility of genetic control over various enzymic conditions affecting the capacity for metabolizing ethanol, little attention has been given to a systematic study of the genetics of ethanol drinking behavior itself. Rodgers & McClearn (1962) have estimated that 97% of the variance occurring in drinking behavior is due to genetic factors. They have reported the establishment of stable strain differences, with regard to alcohol preference, in the mouse and have been able to demonstrate systematic manipulation of the genotype.

The assumption upon which the present study is based is simply that, if a strong genetic determinant of a behavior exists, animals that are close in biological "kinship" should be more alike with respect to that behavioral variable than animals whose "kinship" is more remote. If this assumption is true and if alcohol consumption is genetically determined, the magnitude of correlation coefficients describing the relationship between alcohol consumption of pairs of animals should vary directly with the degree of biological kinship existing between the animals involved.

METHOD

The purpose of the present experiment was to provide circumstances under which the relationship between kinship and the degree of alcohol selection behavior in rats could be examined. The precedent for this design has been well established by research involving other variables now fairly well accepted as being strongly related to genetic factors (e.g., IQ, physical height, weight, etc., in humans).

The design of the present study provided circumstances under which correlation coefficients could be derived between a parent group of hooded rats and (1) their first-generation offspring, (2) their second-generation offspring, as well as between (3) littermates and (4) half siblings.

Experiment 1

The 72 male and 108 female hooded Long-Evans rats that served as the P_1 generation were obtained from two commercial laboratory animal suppliers in order to minimize the possibility of an uncontrolled genetic relationship among the animals.

The P_1 group was exposed to a 7% (v/v) alcohol solution, water, and food ad lib for 2 weeks. Forty male and 60 female rats were selected on the basis of selective alcohol consumption during this 2-week period. The 100 animals were selected such that there was a large interanimal variability and an approximately rectangular distribution of relative alcohol consumption. For the pool of animals, 20 "families" of two male and three female rats each were established such that the members of each "family" exhibited similar degrees of relative alcohol consumption. That is, the two highest EtOH-drinking males and three highest EtOH-drinking females made up the first "family"; the next five highest EtOH-drinking animals made up the second "family"; and so on down to the "family" consisting of the five lowest EtOH drinkers. In each "family" one male rat was mated with one female and the other male was mated with the remaining two females. One of these latter females was designated as Sf, and the other two females were designated as Pf.

One animal of each sex was selected randomly from each litter to form the first filial generations (F_1 and F_1S). When these offspring reached 90 days of age, their relative alcohol consumption was tested (N = 34 pairs Pf, N = 17 pairs Sf). The offspring of the Pf-Pm mates were mated with nonrelated members of the same "family." In this manner, it was possible to produce a second generation containing animals whose grandparents had known and controlled levels of relative alcohol selection. The second generation offspring (F_2), one animal of each sex from each litter, were tested to determine relative alcohol consumption at 90 days of age (N = 25 pairs). Data were thus collected for animals having all degrees of biological kinships described above.

Experiment 2

Due to the apparently inconclusive results of the original study, a second study was carried out but was restricted to animals of direct lineage (see Fig. 1). In Experiment 2, correlation coefficients could be calculated between a parent group and (1) first generation offspring, (2) second generation offspring, and (3) between littermates.

Forty male and 40 female Long-Evans rats were obtained from two animal distributors to serve as the P generation. Subsequent to a 2-week test for relative alcohol consumption, 24 pairs of animals were selected for breeding. The "families" were selected, as before, on the basis of similarity of alcohol consumption ratios. A "family" in Experiment 2 consisted of two males and two females, with the members of each family exhibiting similar alcohol consumption ratios. The F_1 generation was tested for alcohol consumption at 90 days of age (N = 16 pairs) and then mated with nonrelated members of the same "family." Their offspring were then tested for alcohol consumption at 90 days of age (N = 15 pairs).

All animals were individually housed in standard rat cages for all periods except during breeding and rearing periods, when the animals were

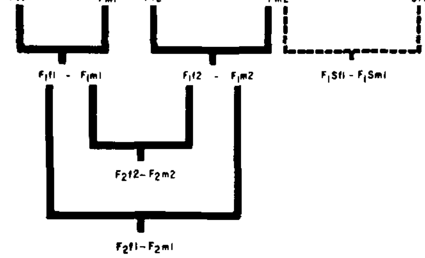

Fig. 1. The breeding schedule for animals involved in the present research. The groups joined by the broken line (upper right) were used only in Experiment 1.

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Table 1
Correlation Coefficients for Two Generations Based on Alcohol Consumption Ratios (Experiment 1)

Degree of Biological Kinship

Littermates

Comparison	r
F_1-F_1	.405***
F_1P-F_1P	.442***
F_1S-F_1S	.338
F_2-F_2	.012

Parent-First Generation

Comparison	r
P-F_1	.232
P-F_1P	.072
P-F_1S	.745***
F_1-F_2	–.058
P-F_1m*	–.029
P-F_1f	.100
P-F_1Sm	.548**
P-F_1Sf	.704***
F_1-F_2m	–.020
F_1-F_2f	–.193
Pm-F_1	.078
Pm-F_1m	–.046
Pm-F_1f	.159
Pf-F_1	.096
Pf-F_1m	–.036
Pf-F_1f	.208
Pm-F_1S	.741***
Pm-F_1Sm	.522**
Pm-F_1Sf	.709***
Pf-F_1S	.746***
Pf-F_1Sm	.627***
Pf-F_1Sf	.688***
F_1m-F_2	–.398
F_1m-F_2m	–.131
F_1m-F_2f	–.378
F_1f-F_2	.167
F_1f-F_2m	.033
F_1f-F_2f	.273

Parent-Second Generation

Comparison	r
P-F_2	.255
P-F_2m	–.006
P-F_2f	.340
Pm-F_2	.400
Pm-F_2m	–.002
Pm-F_2f	.290
Pf-F_2	.379
Pf-F_2m	–.008
Pf-F_2f	.359

Half "Sibs"

Comparison	r
F_1P-F_1S	.424
F_1Pm-F_1S	.112
F_1Pm-F_1Sm	.576
F_1Pm-F_1Sf	.378
F_1Pf-F_1S	.344
F_1Pf-F_1Sm	.242
F_1Pf-F_1Sf	.312
F_1P-F_1Sm	.218
F_1P-F_1Sf	.519**

**Lowercase f and m designate female and male; **p < .05; ***p < .01.*

housed in breeding cages. Animals had ad lib food and water at all times. During the 2-week test periods, the animals were given a choice of 7% (v/v) ethanol solution and water.

RESULTS

The index of relative alcohol consumption was expressed as a ratio of the volume (milliliters) of 7% (v/v) EtOH solution consumed to the total volume (EtOH plus H_2O) of liquid drunk daily. This ratio has been designated as E/T (ethanol/total). The index of relative alcohol consumption was calculated from fluid consumption data collected daily from all animals during 2-week test periods.

Data collected from animals bearing all degrees of biological kinship (P-F_1; P-F_2; littermates; and half siblings in Experiment 1) were arranged in pairs for the computation of correlation coefficients (Pearson r). Average E/T values for two animals were used as single data points where appropriate; in all other cases, data were based on individual values. All coefficients calculated from the data are presented in Table 1.

The correlation coefficients resulting from Experiment 1 present an erratic picture. E/T data collected from animals bearing identical degrees of biological kinship vary enormously. For example, those correlations which involve a parent/first-generation offspring level of kinship yield low positive coefficients for P-F_1 animals, low negative coefficients for F_1-F_2 animals, and highly significant positive correlations for the P-F_1S animals. Consider also the coefficients calculated from data collected from littermates. The F_1-F_1 comparison yielded an r = .405 (p < .01), while the F_2-F_2 comparison yielded an r = .012 (n.s.). Clearly, these data do not reflect a consistent direct variation between magnitude of correlation and degree of biological kinship.

Because of the inconclusive nature of the data collected in Experiment 1, Experiment 2 was undertaken. The correlation coefficients resulting from Experiment 2 are presented in Table 2.

The coefficients resulting from Experiment 2 do very little to alter the picture which existed subsequent to the completion of Experiment 1. The direct relationship expected to exist between degree of kinship and magnitude of the rs describing the degree of covariation in the animals' drinking behavior again fails to appear. The general results of Experiment 2 are contradictory to any reasonable theoretical expectation because most of the obtained coefficients, although not significantly different from zero, are *negative*. Those few coefficients which do reach sufficient magnitude to exceed at least the .05 rejection level are *all negative* and all result from the pairing of data points collected from animals bearing the *least* degree of biological kinship.

The results of the two experiments, when considered separately, seem to present two chaotic, but somewhat complementary, pictures; when taken collectively, however, a possible explanation for the erratic data pattern may emerge. The reader's attention is called to Fig. 2.

Figure 2 shows a frequency distribution of all 79 correlation coefficients calculated from data collected in both experiments. Examination of this distribution indicates that its resemblance to the normal distribution of a random

Table 2
Correlation Coefficients for Two Generations Based on Alcohol Consumption Ratios (Experiment 2)

Degree of Biological Kinship

Littermates

Comparison	r
F_1-F_1	–.103
F_2-F_2	–.081

Parent-First Generation

Comparison	r
P-F_1	.220
F_1-F_2	.125
P-F_1m*	–.225
P-F_1f	–.071
F_1-F_2m	.214
F_1-F_2f	.066
Pm-F_1	–.244
Pm-F_1m	–.296
Pm-F_1m	–.032
Pf-F_1	–.184
Pf-F_1m	–.138
Pf-F_1f	–.110
F_1m-F_2	.273
F_1m-F_2m	.164
F_1m-F_2f	.204
F_1f-F_2	–.099
F_1f-F_2m	.088
F_1f-F_2f	–.035

Parent-Second Generation

Comparison	r
P-F_2	–.640†
P-F_2m	–.866**
P-F_2f	–.356
Pm-F_2	–.680†
Pm-F_2m	–.844**
Pm-F_2f	–.879***
Pf-F_2	–.579
Pf-F_2m	–.074
Pf-F_2f	–.429

**Lowercase f and m denote female and male; **p < .05; ***p < .01.*
†The relatively large coefficients do not reach significance because of the relatively low Ns involved.

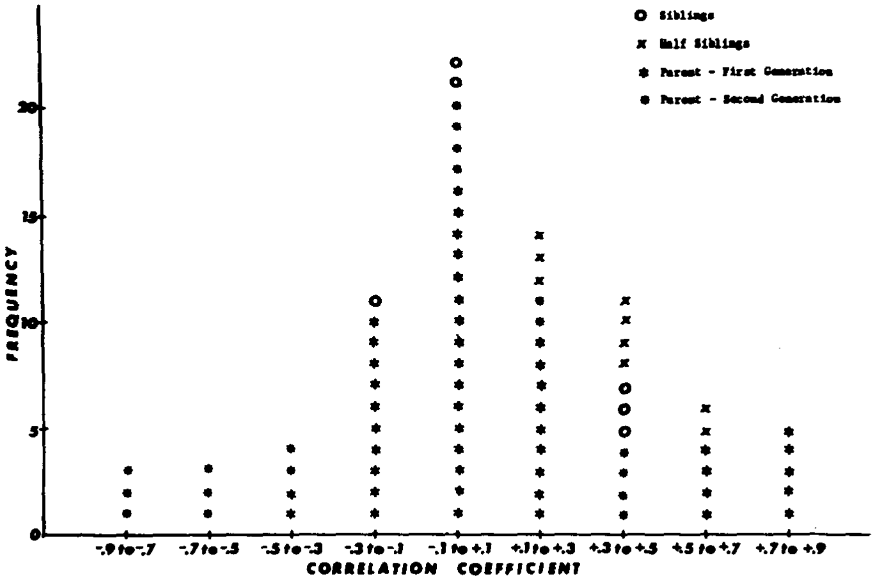

Fig. 2. The frequency distribution of all values of r calculated from both Experiment 1 and Experiment 2.

variable is more than superficial. By actual computation, the distribution shown in Fig. 2 was found to have a mean of .082 and a standard deviation of .37. These empirical data closely approximate a hypothetical *null* distribution of correlation coefficients (with $\mu = 0$ and $\sigma = .37$).

An examination of the distribution shown in Fig. 2 via the chi-square goodness of fit test yielded $\chi^2 = 13.3$ (df = 7; $p > .05$), indicating that the obtained distribution of values of r form a distribution which is not significantly different from a normal Gaussian distribution with $\bar{X} = 0$ and S = .37!

The number of significant (.05) coefficients actually obtained from the empirical data was 15. This considerably exceeds the number one would expect to occur by chance among 79 randomly calculated correlation coefficients (i.e., ≅ 4). The reader's attention, however, is called to the fact that the rs calculated with the data collected in these two experiments are *not* independent and that many of the significant correlations reported involve computations with data collected from overlapping sets of animals (e.g., the P-F_1 S combinations in Experiment 1 and the P-F_2 combinations in Experiment 2).

DISCUSSION

If it is assumed that the tendency for an animal to select and consume an EtOH solution is related to biological kinship, it seems reasonable to suppose that animals with greater similarity of kinship should also exhibit greater similarity in ethanol consummatory behavior than would animals bearing lesser degrees of biological kinship. If there is such a covariation between the tendency to select and consume ethanol and the degree of kinship among animals, it should be able to be detected by the statistical method of correlation. There should exist a direct relationship between the magnitude of correlation, calculated from indices of alcohol consumption, and the degree of biological kinship existing among the animals involved.

The results of the present study fail to yield any evidence in support of the contention that the relative alcohol consumption in the rat, as measured by E/T ratios using 7% v/v ethanol, is dependent upon the biological kinship of the animals involved. Instead of observing the systematic variation in the magnitude of correlation as a direct function of degree of biological kinship, the results of the studies presented here seem to make a much more substantial case for *virtual independence* of the two variables involved. The correlation coefficients resulting from the two studies described herein, involving a total of six generations of rats, bear striking similarity to a set of data points randomly selected from a normal Gaussian population of correlation coefficients with $\mu = 0$.

The only event observed during the course of this research which would appear to deviate markedly from chance expectancy is the fact that the first experiment yielded more positive correlations, whereas the second experiment yielded a preponderance of negative correlations. The difference in direction of these two sets of correlations was significant ($U = 193$, $N_1 = 50$; $N_2 = 29$, $z = -5.4$, $p < .01$) but could in no way be attributed to kinship variables.

If the results of Experiment 1 are viewed independently, the reasonable conclusion would have been to surmise that a general low positive but nonsignificant correlation existed between drinking behaviors of the experimental animals, but the degree of correlation does not vary systematically with degree of kinship. The results of Experiment 2 seemed to produce almost a mirror image of Experiment 1, with generally low, nonsignificant negative correlations appearing and the magnitude of the correlation being again apparently unrelated to biological kinship. Taken collectively, the results of both experiments paint what would appear to be an almost perfect picture of random variation among the coefficients calculated.

The data reported here do not directly contradict previous findings or theories regarding the possibility of a kinship influence in the etiology of human alcoholism or even in the control of ethanol ingestion exhibited by lower animals because of the obvious differences in experimental Ss and methodology. A rather convincing single example of a case (using specific animals and a specific method) in which kinship factors seem unrelated to ethanol selection and consumption in experimental Ss is, however, offered for the reader's consideration.

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